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Ebolavirus: Implications for the Clinical Laboratory

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EDITORS NOTE: *Recently we have all been exposed to two connected but individually terrifying diseases: scientific illiteracy and the inability of scientists to speak intelligible English (One note, I will use English throughout as it is the common language of the United States but will mean any common language of any country.) The combination of poor speakers and poorly prepared listeners has allowed an unreasoned and unreasonable fear to spread over this country. It is time for those who can speak coherently and factually to take the place of those who cannot. The Editorial Board of Clinical Laboratory Science hopes that the information in this attachment will do more good if it were to be electronically distributed instead of waiting for a routine publication. We felt that it was important that all of our members, scientists all by education, would value the information. We ask that you work to correct the misinformation and muddled thoughts that are currently flooding our airwaves by using it with your families, friends and colleagues.*

For example, in answer to the question " Do you believe in quarantining people?" one noted scientist said," If she were my patient, I would not recommend it." What ever happened to "no"? Another question, "Could the Ebola virus mutate and become airborne? And the answer was, Well, it is mutating quite rapidly now. Since the 1970s when the Filoviridae Family was first investigated, there has never been a single suspicion that any of them could be transmitted to humans in this fashion. The currently held theory is that humans lack a receptor required for any of these viruses to be transmitted in this fashion.

The clinical laboratory is a major source of science used in medicine. We need to start explaining the truth to our families and friends. We need to explain how this virus is transmitted. We need to explain about the inability of the virus to be transmitted by anyone prior to the onset of symptoms. We need to explain why containing at its source is the only logical next step. The Nobel Peace Laureate organization, Médecins Sans Frontières, has been pleading for months for supplies, equipment, and people to work with them in West Africa. How sad it is that we only think to help others when we are confronted with the disease on our own soil.

In the absence of clearly thought out, correctly worded information from the various medical establishments and from our national and state governments, it is important that we identify ourselves as the holders of both the knowledge and the ability to explain that knowledge to the general populace.

Our two authors are highly respected in the fields of infectious disease and public health. They have written and lectured widely on various topics over the decades and both are currently involved with several governmental agencies and hospitals across the country. The information that forms the background of their manuscripts comes from the World Health Organization, the Médecins Sans Frontières,_US Centers for Disease Control, the National Institutes of Health and more.

INTRODUCTION

A minor river valley virus in central Africa has now grabbed the world's attention. West of the Great Rift Valley, North of the Kinshasa Highway, a small tributary of the Congo River, the Ebola River is the

namesake source of this latest attention-grabbing infectious disease. Past Ebola outbreaks have been documented in the following locations: Democratic Republic of the Congo (DRC), Gabon, South Sudan, Ivory Coast, Uganda, Republic of the Congo (ROC), and South Africa (imported).[1] The virus has now migrated from its natural location in central sub-Saharan Africa across the African Transition-Zone to West Africa. The exact mechanism for doing this after all this time is unknown but may be related to bat migration.[2] For this outbreak, the World Health Organization (WHO) has calculated a likely "epicenter" including a small province in south Guinea, two in Sierra Leone and another in Liberia. (See Figure 1)

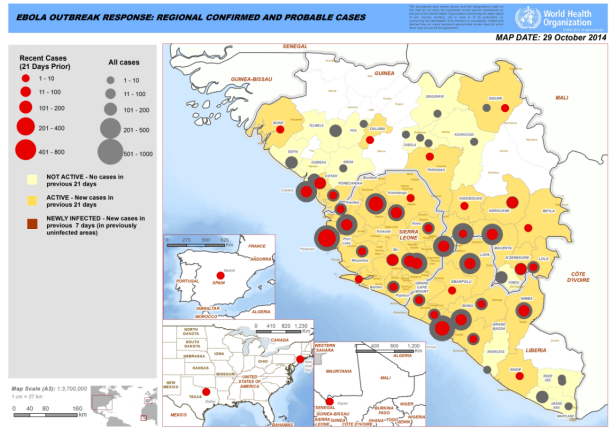

Figure 1. World Health Organization Map of Ebola Outbreak October 29, 2014

The original viruses were isolated in 1976. [Table 1] The nature of the virus has been worked out. [Table 2] Specifically the virus is enveloped, helical, and filamentous with a cross-striated nucleocapsid and has extensive branching. The virus measures 80 nm (dia) X 970-1200 nm (long) although some forms have been described as 14,000 nm in length. Typically the RNA is 18.8 kb coding for 7 products and with a mutation rate of 0.46 x 10^{-4} to 8.21 x 10^{-4} nucleotide substitutions/site/yr.[3]

VIRAL SUBTYPES

EBO-R and EBO-Z had a common ancestor as recently as 1960 and Marburg and EBO-S had a common ancestor somewhere between 1164-1414. There was a family divergence approximately 10,000 years ago from the paleoviruses of hampsters and voles. In general, mammals have been dealing with something like Ebola viruses for the last 10 million years.[4]

Table 1. Nomenclature of Ebola River Hemorrhagic Fever Variants

Year	Subtype	Mortality
1976	Ebola Sudan (EBO-S) (Nzara, Sudan)	53%
1976	Ebola Zaire (EBO-Z) (Yambuku, Zaire){DR Congo}	88%
1989	EBO-R, (Reston) Imported Monkeys (Philippines)	0%
1994	EBO-CI Chimp Autopsy (Tai Forrest, Côte d'Ivoire)	?
2007	EBO-B Bundibugyo Ebola Virus (A district in Uganda)	1st 100 victim outbreak

Table 2. Classification of Ebola River Virus

Taxon	Specific
Group	Group V ((-)ss RNA)
Order	Mononegavirales
Family	Filoviridae
Genus	Ebolavirus
	Cuevavirus
	Marburgvirus

Beginning in epidemiologic week #51 of 2013, likely in Guinea with "Patient Zero" the epidemic had spread to Sierra Leone and Liberia by Sept 29, 2014. The likely patient zero was named Emile Ouamouno and he died on December 6, 2013. Subsequently, Emile's 3-year-old sister, his mother, and his grandmother all succumbed to the disease by January 2014, leaving only his father behind.[5]

According to the WHO over 4500 case of Ebola have been diagnoses with a mortality rate of 50% by the middle of September with a projection of over 20,000.[6] (Figure 2)

Overall, it is not entirely known exactly how primates contract the filoviruses in nature. We do know that secondary cases of Ebola have come about and can come about as the result of human contact with blood, organs, semen and other bodily secretions (feces, saliva, sweat, vomit) via mammals (humans, bats, monkeys and apes). While it has been detected

in breast milk, there has been no known transmission via this route. Similarly Ebola is NOT known to be transmitted via air, water or food or via mosquitoes or other insects.[7] While there has been some research indicating possible respiratory transmission in nonhuman primates, this has never been observed in humans and there is some indication that this may be due to lack of receptors.[8]

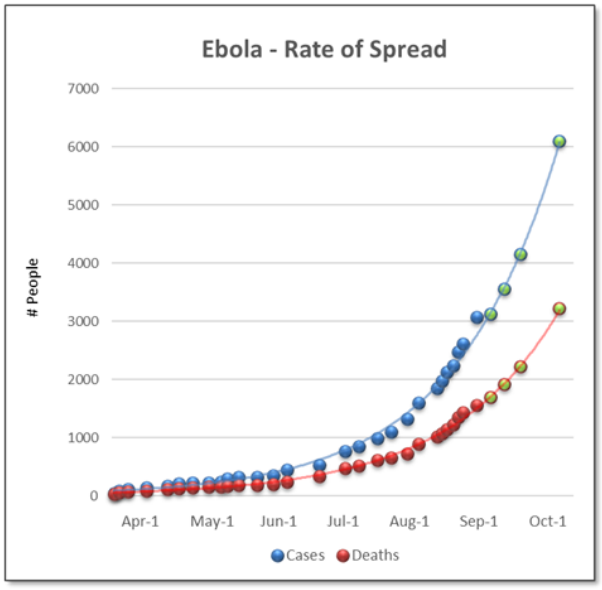

Figure 2. World Health Organization of Case Rate as of October 1, 2014

EPIDEMIOLOGY

Many health experts suspect that the initial cases in many Ebola outbreaks may begin with people eating or handling Ebola infected animals. "Bush meat" is often a delicacy and includes animals such as rodents and wild pigs, as well as fruit bats and primates.[9]

Actual viral parasitism at the cellular level involves attachment of the virus to various target cells such as endothelium, macrophages, dendritic cells, monocytes or hepatocytes. Attachment is followed by macropinocytosis and endosome dissolution resulting in the virus ending up in the cell cytoplasm. Once there and not in an endosome, the virus rapidly overwhelms the protein synthesis of the host cell resulting in rapid viral replication. In endothelial cells a viral-coded glycoprotein (GP) forms a three-element complex binding the virus to the interior surface of the blood vessel. There is also a secreted glycoprotein (sGP) that interferes with signaling PMNs and hence contributes to immune invasion.[10] This results in PMNs carrying the Ebola virus to distal parts of the body (lymph nodes, liver, lungs and spleen). Viral particles and cell damage resulting from viral budding and release chemical signals (TNF-a, Il-6, Il-8) that initiate the fever and other signs of infection. Eventually there is loss of vascular integrity and hypovolemic shock.[11] (Table 3)

It is important to note that the ability to transmit Ebola occurs at the onset of symptomology but diagnostic tests are generally useful a few days after the first symptoms and would include Antigen-Capture ELISA, IgM ELISA, RT-PCR and virus isolation. Later during the course of the infection or in recovery, IgM Antibodies or IgG Antibodies are useful, and still later (retrospective or necropsy), Immunohistochemistry, RT-PCR and virus isolation work well.[12] Rapid diagnostic tests are being rushed via FDA "fast track" approval process in which a single drop of blood can be utilized much like a pregnancy test and yield a result in 10-15 minutes. It's important to remember that these tests must gain federal approval before being used in the current outbreak in West Africa or elsewhere.

TREATMENT OPTIONS

On the treatment side, the standard approach is supportive therapy; although newer therapies, several as yet fully examined, are being used. (See Table 4) The now well-known ZMAPP is a set of three monoclonal antibodies humanized by genetic engineering. Previously, it had never been given to humans but based on a positive experience in monkeys, it was used on humans.[13] The numbers are currently insufficient to determine if it was effective in these cases. The nucleoside analogues are currently being studied in mice.

One serious issue with ZMAPP and other intervention of this type is called the "Cold-Chain" problem in that these agents must be kept under strict temperature management during preparation, transport and storage, a difficult to impossible characteristic in 3rd world situations.[14]

OTHER IMPLICATIONS

Assuming a "low Ebola" scenario, the World Bank estimates the lost to GDP for West Africa as a whole

Table 3. Signs and Symptoms of Ebola Grouped by Significance and Frequency. Those in **GREEN** are most likely to appear early in the patient's awareness of the disease **and** those in **BLUE,** later.

Frequent	%	Common	Infrequent	Rare
Fever	90 - 100	Vomiting	Chills	CNS involvement
Headache	40 - 90		Pharyngitis	
Myalgia	40 - 80		Loss of appetite	
Malaise	75 - 85	Hepatic damage	Hematemesis	
Dry and Sore throat		Renal failure	Abdominal pain	
Non-bloody diarrhea	80 - 85	Thrombocytopenia	Lymphopenia	
DIC and fibrinolysis	71 - 78	Transaminase elevation	Hiccups - very poor prognostic sign	
Chest pain	- 83% of EBO-S	Hyperamylasemia	Maculopapular rash	
		Terminal shock	Chest pain - uncommon in EBO-Z	

to be at least $2.2 billion in 2014 and $1.6 billion in 2015. Depending on how successful the world's response to this outbreak is, the "high Ebola" estimates suggest a loss of $7.4 billion in 2014 and $25.2 billion in 2015.[15]. These countries cannot sustain this impact. One example of the societal destruction is the number of children orphaned and refused family support as some people see them "witches" for having survived.

As Manuel Fontaine, UNICEF Regional Director for West & Central Africa, who just returned from a two-week visit to Guinea, Liberia and Sierra Leone reported, "Thousands of children are living through the deaths of their mother, father or family members from Ebola. These children urgently need special attention and support; yet many of them feel unwanted and even abandoned. Orphans are usually taken in by a member of the extended family, but in some communities, the fear surrounding Ebola is becoming stronger than family ties".[16]

The current outbreak is in countries which have at best inadequate and in some locations no public health infrastructure, economic stability and/or governance to effectively deal with these issues. The World Health Organization and the International Health Regulations currently lack sufficient resources due to budget cuts, staffing issues and concerns over agency disputes and intergovernmental issues.[17]

PERSONAL PROTECTIVE EQUIPMENT

While Personal Protective Equipment and measures would be primarily for those who care for an Ebola patient(s) or those who travel to the affected areas of West Africa, medical laboratorians should be cognizant of and follow CDC guidelines for healthcare workers (current as of October 20, 2014).

This guideline contains the following key principles:

1. Prior to working with Ebola patients, all healthcare workers involved in the care of Ebola patients must have received repeated training and have demonstrated competency in performing all Ebola-related infections control practices and procedures, specifically in donning/doffing proper PPE.
2. While working with and in PPE, healthcare workers caring for Ebola patients should have no skin exposed.
3. The overall safe care of Ebola patients in a facility must be overseen by an onsite manager at all times, and each step of every PPE donning/doffing procedure must be supervised by a trained observer to ensure proper completion of established PPE protocols.

For specific guidance on healthcare worker PPE and the importance of training, practice, competence, and observation, visit http://www.cdc.gov/ vhf/ ebola/hcp/ procedures-for-ppe.html.

For the interim guidance document to laboratory workers on specimen collection, transport, testing, and submission, visit http://www.cdc.gov/niosh/topics/ebola /healthcare.html#labspecific guidelines.

Many problems have arisen through the hysteria created in news reports. Not the least of these have involved testing and hazardous material handling. Obtaining the specimens from a patient clearly would expose the phlebotomist to patient secretions that could easily transmit the virus from a symptomatic patient.

Current CDC guidelines as to PPE need to be followed without exception. Pre-symptomatic patients are just like all our other patients and the procedures and protocols we follow every day are appropriate. Transporting specimens (on site) should follow the same guidelines as apply to other specimens we have except NO PNEUMATIC TUBES for suspected Ebola patient specimens.[18]

Table 4. Appropriate Therapies. Notice that all of the currently used supportive therapy require significant laboratory utilization.

SUPPORTIVE THERAPIES	ATTEMPTED THERAPIES	ANTICIPATED THERAPIES
Balancing fluids and Electrolytes	Monoclonal antibodies from Ebola survivors	TKM-EBOLA RA interference (ABI-7537) research stopped due to budget cuts
Maintaining O2 status	Promising vaccine (guinea pigs)	Favipiravir
Maintaining blood pressure	Nucleoside analogue inhibitors of S-adenosylhomo cytsteine hydrolase	selective estrogen receptor modulators Clomiphene - Toremifene BCS-4430 – ST- 383
Replacement of blood products treatment of complicating infections	ZMAPP	

Wherever possible try to collect specimens in plastic tubes/vials so as to minimize the hazards of breakage. Some mail carriers have announced policies that would block receipt of "confirmed" Ebola patient blood, but not "suspected" patient specimens. Our advice is to follow the CDC as well as your state health department protocols for access to state lab testing or testing at CDC. It is important to be aware of the significant consequences of releasing a patient who has Lassa fever, Typhoid or Malaria simply because a test for Ebola is negative.

Another hidden implication for the clinical laboratory will be the type of impact the ongoing crisis may have for education. MLS (CLS) and MLT programs are typically required to provide clinical education to their students as part of any NAACLS approved program. These clinical rotations or experiences are an integral part of any student's learning to prepare for certification and/or licensure in the profession. These experiences are also a vital component for the real world transition of students to future employees in medical laboratories. In Texas, some hospitals are adopting standard operating procedures that would limit or remove nursing, respiratory care, and other direct-patient care training if an Ebola or suspected Ebola patient enters there hospital. It is likely that this limitation or ban on student clinical training should be anticipated for the MLS or MLT student. In this possible (and usually rapid) scenario, educational programs may be required to quickly respond to providing clinical training on site at their educational institution, via simulation, or other appropriate measures.

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